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PLANT ANALYSIS FOR NUTRIENT ASSAY OF NATURAL WATERS

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Wisconsin University

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Plant Analysis for Nutrient Assay of Natural Waters

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16. Abstract

Plant analysis has been developed as a procedure for evaluating nutrient supplies and growth-limiting nutrients for nuisance macrophytes in lakes and streams. Plant analysis is based on establishing in index segments the critical concentration (minimum plant concentration for maximum yield) of each essential nutrient element likely to limit growth of nuisance macrophytes. Critical concentrations for nitrogen, phosphorus, sulfur, calcium, magnesium, potassium, iron, manganese, zinc, boron, and molybdenum were established in appropriate index segments of Elodea occidentalis. The critical copper concentration was estimated. Critical concentrations for several elements also were established in Ceratophyllum demursum.

To evaluate plant analysis as an assay procedure, index segments of Elodea and Ceratophyllum were routinely collected from Wisconsin lakes, analyzed, and the analyses were compared with the critical concentrations for indications of nutrient deficiency. Nitrogen, phosphorus, calcium, and copper were at or close to critical levels in one or more lakes. Neither nitrogen nor phosphorus seemed to be a general growth-limiting nutrient in the lakes sampled. (EPA)

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ABSTRACT

Plant analysis was developed as a relatively simple procedure for evaluating nutrient supplies and growth-limiting nutrients for nuisance macrophytes in lakes and streams. Plant analysis requires establishing in index segments of the macrophytes the critical concentration (minimum plant concentration for maximum yield) of each essential nutrient likely to limit growth. Critical concentrations for nitrogen, phosphorus, sulfur, calcium, magnesium, potassium, iron, manganese, zinc, boron, and molyldenum were established in appropriate index segments of Elodea occidentalis. The copper critical concentration was estimated. Critical concentrations for nitrogen, phosphorus, and several other elements were established in Ceratophyllum demursum.

To evaluate plant analysis, samples of Elodea and Ceratophyllum were routinely collected from Wisconsin lakes, analyzed for essential nutrients, and the analyses were compared with the critical concentrations for indications of nutrient deficiency. A growth-limiting role of an element in a lake was indicated by plant concentrations below the critical level. Nitrogen, phosphorus, calcium, and copper were at or close to critical levels in one or more lakes. Neither phosphorus nor nitrogen seemed to be a general growth-limiting nutrient in the lakes sampled. The most unexpected result was an indication of copper deficiency in several lakes.

From the extensive nutritional experiments to establish critical element concentrations, a synthetic nutrient medium for general macrophyte culture was developed.

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SECTION I

CONCLUSIONS

The primary conclusions from studies on this project can be summarized as follows:

1. Plant analysis is a relatively simple and useful procedure for evaluating nutrient supplies and growth-limiting nutrients for nuisance macrophytes, and probably for other obnoxious plants as well.

2. Plant analysis is most reliable as a diagnostic technique when based on the index segments established in these studies (the first and second one-inch segments of stems and laterals) rather than on entire plants.

3. Establishing critical concentrations for most of the essential elements in Elodea occidentalis makes it possible to use this species as a general bioassay organism in evaluating whether any of these elements become growth-limiting in lakes or streams.

4. Evaluations of nutrient supplies in Wisconsin lakes indicated neither nitrogen nor phosphorus was a general limiting nutrient in the lakes sampled.

5. The element most likely to limit macrophyte growth varied in different lakes. Evidence was obtained that nitrogen, phosphorus, calcium, and copper were limiting, or were close to growth-limiting, in different lakes.

6. Although confirming evidence is required, the results indicated copper deficiency is common in soft-water, infertile northern Wisconsin lakes.

SECTION II

RECOMMENDATIONS

Simple, reliable techniques are urgently needed for nutrient assay in lakes and streams. This project was an initial effort to develop and apply plant analysis as a nutrient assay. The results suggest plant analysis is a promising technique. However, additional work is recommended to verify this promise and to further refine the technique.

Critical concentrations and index segments should be established in macrophytes other than the two species used in the work on this project. It would be desirable to know if the critical concentrations for Elodea occidentalis and Ceratophylum demursum were generally applicable to macrophytes. It also seems desirable to develop plant analysis using aquatic organisms other than macrophytes. A filamentous, green alga which is responsible for nuisance conditions seems a suitable possibility.

Determining whether analysis for the total concentration of an element or for an extracted fraction more reliably correlates with yield also would be desirable. In agricultural applications, for some elements and in some species extracted fractions have been found more reliable than total concentrations.

Further evaluations of the plant analysis technique should be made using the organisms and critical concentrations established on this project. This would involve additional samplings of Elodea and Ceratophylum from Wisconsin lakes differing in fertility. Further tests with the assay species isolated in floating, porous baskets and sampled through the summer, as initiated on this project, also seem highly desirable. An effort should be made to obtain samples of natural populations of macrophytes from lakes in other parts of the United States in which deficiencies of specific elements have been indicated. Analyses would be compared with critical concentrations established on this project to verify the suggested deficiencies and the usefulness of the plant analysis technique.

Results on this project suggested macrophytes in some northern Wisconsin lakes are copper deficient. Copper becomes growth limiting. This must be verified. If true, the copper requirements of other nuisance aquatic plants should be studied.

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Plant analysis is one of several techniques suggested or in use for nutrient assay in natural waters. This seems a highly appropriate time to evaluate these various techniques and to compare them with plant analysis. Chemical analyses of water samples, ^{14}C uptake following nutrient enrichment, the Provisional Algal Assay Procedure, and enzyme assays such as the measure of phosphorus deficiency in plants by phosphatase activity are among the techniques that should be compared.

SECTION III

INTRODUCTION

Aquatic plants, both algae and angiosperms (macrophytes), are responsible for nuisance conditions when excessive growths of the organisms develop in polluted lakes and streams. One approach to the control of these undesirable growths is to reduce supplies of an essential nutrient element to growth-limiting levels.

Considerable evidence indicates that nitrogen and phosphorus are most likely to become limiting for plant growth in aquatic environments, and most investigators favor phosphorus as the primary limiting nutrient. However, evidence obtained by Gerloff and Skoog (1957) suggested that nitrogen rather than phosphorus limited growth of the blue-green alga Microcystis aeruginosa in several southern Wisconsin lakes. In a recent report, (Ryther and Dunstan, 1971) nitrogen was considered to become limiting for algae in coastal marine waters of northeastern United States. Data obtained by Goldman (1960, 1964) indicated that in some lakes trace element supplies limit aquatic plant growth.

Reliable assays of nutrient supplies in lakes and streams relative to plant needs would be highly useful to engineers, water chemists, biologists, and others who must predict and assess the effectiveness of suggested pollution control measures. Various procedures have been proposed and used for nutrient assay, for example chemical analyses of water samples and enrichment bioassays involving growth or photosynthesis of aquatic organisms following additions to water samples of nutrient elements that might be growth limiting (Gerloff, 1969). There are problems associated with both approaches and neither has been developed to a degree that has led to acceptance as a general diagnostic procedure. For example, it is difficult to interpret water analyses in terms of concentrations at which specific elements become growth limiting (Lee, 1969; Lund, 1969). Enrichment assays, in addition, are subject to errors associated with extending data obtained with isolated samples to evaluations of nutrient supplies in large bodies of water (Gerloff, 1969). As an alternative, a bioassay based on plant rather than water samples has been developed (Fitzgerald, 1969). The amount of orthophosphate extracted from plant samples with boiling water and the uptake of NH_4-N in the dark correlate with the

adequacy of phosphorus and nitrogen nutrition, respectively, of the plants sampled.

Plant or tissue analysis is a technique for nutrient assay that has found widespread application in assessing the nutrient status of soils for the production of agricultural and horticultural crops (Bould, et al., 1960; Reuther, 1961; Smith, 1962; Chapman, 1966). Plant analysis is based on the observation that the concentration of any essential element in a plant can vary over a considerable range, and that a primary factor determining the concentration in a healthy plant is the availability of the element in the environment. The essential relations on which tissue analysis is based are shown in Figure 1 (Ulrich, 1961).

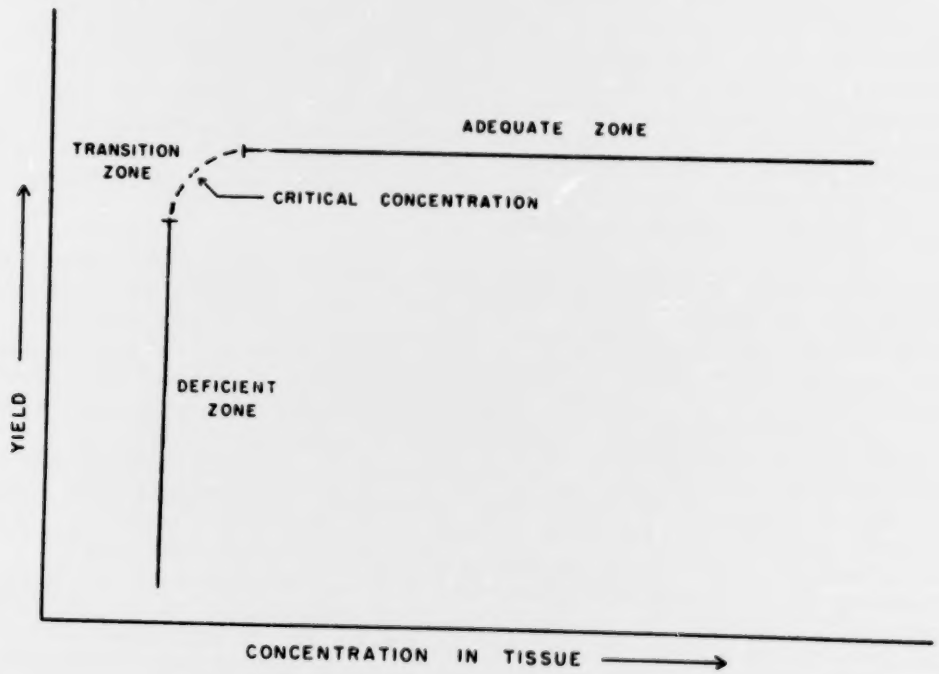

Figure 1. Diagram of the relationship between plant yield and the concentration of an essential element in specific plant parts (From Ulrich, 1961).

The nearly vertical part of the hypothetical curve is termed the "Deficient Zone"; here, plant yield is increasing markedly, but the concentration in the organism is changing very little. In the horizontal part of the curve, organism concentration of the element is increasing, but yield is not; this is the "Adequate Zone", or, more commonly, the "Zone of Luxury Consumption". The "Transition Zone" is that part of the curve between the zones of deficiency and adequacy. Successful application of tissue analysis depends on establishing for a species the critical concentration for each element of interest. The critical concentration is that content which is just inadequate for maximum growth.

Application of the plant analysis technique in evaluating nutrient supplies for aquatic plants would require establishing in laboratory experiments the critical concentration for each potentially growth-limiting essential element in the plant species of interest. The same species then would be collected from lakes and streams, analyzed for various elements, and the concentrations compared with the critical levels. If a plant from the field contains less than the critical concentration of an element, the supply of that element was limiting growth in the environment from which the plant was collected. More growth would result if greater amounts of the nutrient could be absorbed.

The plant analysis technique seems particularly applicable to evaluating nutrient supplies in aquatic environments because of the difficulties in obtaining representative water samples and in interpreting concentrations of elements in terms of potential for plant growth. In plant analysis, aquatic plants become the sampling devices and the concentration of an element in plants reflects all the factors which influenced availability of that element in the environment from which the plants were collected.

The work to be reported was concerned with (1) development of plant analysis as a simple procedure for evaluating nutrient supplies in lakes and streams, (2) testing of the plant analysis technique in nutrient evaluation in representative Wisconsin lakes, and (3) development, through laboratory studies, of an optimum culture medium and general growth conditions for the culture of macrophytes. The experimental work on this project will be presented in three sections corresponding to the objectives mentioned above.

SECTION IV

DEVELOPMENT OF PLANT ANALYSIS AS AN ASSAY OF NUTRIENT SUPPLIES FOR THE GROWTH OF NUISANCE MACROPHYTES

Development of plant analysis for nutrient assay in lakes and streams required that the critical concentrations of all of the essential elements likely to limit plant growth would be established in laboratory experiments for the macrophytes on which the assay was to be based. Elodea occidentalis and Ceratophyllum demursum were selected as the primary test organisms. Both species are abundant and troublesome in Wisconsin lakes. There is a marked morphological difference in the two organisms in that Elodea produces abundant roots while Ceratophyllum does not. In the time available, critical concentrations for all the essential elements except chlorine and copper were established in Elodea occidentalis and for a number of the elements in Ceratophyllum demursum.

EXPERIMENTAL PROCEDURES

The composition of the nutrient medium used in the critical concentration experiments is indicated in Table 1. This is the same culture medium employed in earlier studies (Gerloff and Krombholtz, 1966) except that iron (0.56 ppm) was provided as a chelate of EDDHA (ethylenediamine di-(σ-hydroxyphenylacetate) rather than as FeEDTA. Algae-free cultures were essential. Otherwise, the abundant algae growth which developed made it impossible to interpret the results in terms of the nutritional requirements of the macrophytes. The procedure for obtaining algae-free macrophytes also was described earlier (Gerloff and Krombholtz, 1966).

The most convenient culture vessels were three-liter Florence flasks containing two liters of nutrient medium. The flasks were stoppered and closed except for aeration and exhaust tubes provided with cotton filters in glass-tubing that passed through the stoppers. Each complete assembly was sterilized by autoclaving. The cultures were bubbled continuously with air filtered through cotton and activated charcoal and then enriched to 1% CO_2. All cultures were kept in a constant environment room or growth chambers maintained at approximately 23°C and provided with artificial light of 800-1700 foot candle intensity. The cultures in a

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Table 1. Composition of a modified Hoagland's solution used for the culture of angiosperm aquatic plants.

Salt	Conc.	0.5 M soln. in 1 l final soln. (ml).	Conc. in final solution (ppm)
KNO_3	0.5 M	2.0	N - 42
$Ca(NO_3)_2 \cdot 4H_2O$	"	2.0	K - 47
$MgSO_4 \cdot 7H_2O$	"	0.8	Ca - 40 S - 12.8
KH_2PO_4	"	0.4	P - 6.2 Mg - 9.6
KCl	*	---	Cl - 1.77
H_3BO_3	"	---	B - 0.27
$MnSO_4 \cdot H_2O$	"	---	Mn - 0.27
$ZnSO_4 \cdot 7H_2O$	"	---	Zn - 0.13
$CuSO_4 \cdot 5H_2O$	"	---	Cu - 0.03
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	"	---	Mo - 0.01
FeEDDHA	**	---	Fe - 0.56

* Trace element stock solutions were prepared at 1,000X the concentration of the final solution. One ml of each stock solution was added to each liter of the final culture medium.

** Iron was provided as a chelate of ethylene di(o-hydroxyphenylacetate); 0.56 ppm of Fe was added when the culture medium was prepared and 0.28 ppm 1-2 weeks into the culture period.

particular experiment were inoculated with small sections, approximately 2 inches in length, of the appropriate species removed from continuously maintained stock cultures. Culture periods of 4-5 weeks were necessary to obtain the yields reported.

To develop macrophytes deficient in the essential trace elements (Mn, Zn, B, Cu, and Mo) it was necessary to use standard procedures for reducing environmental contamination, that is in acid-washing culture and media containers, double-distilling water used to prepare culture solutions, and in purifying culture media salts (Hewitt, 1966; Stout and Arnon, 1939).

Inorganic analyses were by quantitative procedures in general use for plant analysis. Total nitrogen was determined by a semi-micro Kjeldahl procedure. Phosphorus determinations were by a vanado-molybdate yellow-complex procedure following dry ashing of oven-dried plant material at 550°C (Jackson, 1958). Potassium analysis was by emission flame photometry of 1 N ammonium acetate extracts of tissue samples. Tissues were prepared for iron analysis by a combined wet-dry ashing procedure which permits ashing at low temperatures. Iron then was determined as a complex with o-phenanthroline. Following dry ashing and acid solution of the residues, calcium, magnesium, zinc, manganese and copper were determined by atomic absorption using a Jarrell-Ash instrument. Boron was determined as a curcumin complex (Johnson and Ulrich, 1959) and molybdenum as a complex with thiocyanate following stannous reduction (Johnson and Arkeley, 1954). Both analyses were on dry-ashed tissues. Analyses for sulfur were by turbidimetric measurements of $BaSO_4$ precipitated in HNO_3-$HClO_4$ digests of plant tissues (Blanchar, Rehm, and Caldwell, 1965).

To minimize contamination, samples analyzed for trace elements and iron were ground with an agate mortar and pestle. When sample size permitted, analyses for the major essential elements were on plant material ground in a Wiley Mill equipped with a stainless steel screen. Otherwise the samples again were ground with a mortar and pestle. In the initial work on this project, critical concentrations were established and evaluated from analyses of entire plants. As in agricultural applications, this proved unsatisfactory, particularly in the collection and analysis of plants from lakes in which nutrient supplies were being evaluated. Large portions of plants too often were unhealthy for reasons other than nutrient deficiency; reduced light for example, and were low in nutrient

concentrations as a result. This could give a false indication that a specific element was growth limiting. Therefore, the critical concentration of each element was established in a plant part (index segment) which more accurately reflected environmental supply than did the entire plant.

Two procedures were employed in determining the critical concentration of nitrogen and phosphorus. One approach was similar to the procedure routinely used in agricultural applications of plant analysis. The plants were grown in nutrient cultures similar in all respects except for the concentration of the element under investigation. Concentrations of that element varied from suboptimal to above optimal. The plants were harvested after a culture period of approximately four weeks, when ranges of growth and of element concentration in the plants were represented. All treatments were at least in duplicate and often in triplicate.

To establish the most suitable index segment, at harvest plant material from each culture was divided into three or four segments: the terminal one inch of growth on main branches and laterals, the second inch of growth, the third inch of growth (in some cases), and the remainder of the plants. Samples were oven-dried to constant weight at 65-70°C, and analyzed for the element under study. A critical concentration was established from curves relating average oven-dry yield and tissue content of the element under study in the plant segments from each treatment of an experiment. The first one-inch portion cut from all main shoots and laterals was found to be the most suitable index segment for elements which are relatively immobile in plants, that is, they are not re-exported from older to younger tissues. The second one-inch was the index segment for mobile elements.

A second procedure was developed for establishing critical concentrations in Elodea. Individual plants were grown in 34 x 20 x 5 cm Pyrex trays containing 1200 ml of the appropriate nutrient medium. Each tray was covered with a glass plate which was sealed to the tray with Apiezon sealing compound (Associated Electrical Industries, Ltd.) after the culture assembly had been autoclaved and inoculated. This was necessary to minimize algae contamination. Two

holes were drilled in each glass cover. Surgical rubber tubing inserted through one hole brought air (filtered and 1.0 percent CO_2 enriched) into the culture. Four capillary air outlets provided vigorous aeration of the nutrient medium. The second hole was plugged with cotton and permitted air to escape. The inoculum for a tray was a 1-1/2 - 2 inch terminal segment of an Elodea main shoot or lateral.

In a specific experiment, some trays contained the complete medium; others contained amounts of nitrogen or phosphorus which would support a maximum rate of growth only for a limited period. Plant growth in the trays was followed by daily measurements of the total length of the plants, both main and lateral shoots. These measurements were made without removing the cover from a tray. From continuous plots of growth in the deficient and the complete cultures, the point at which the nitrogen or phosphorus content of plants in the deficient cultures had been sufficiently reduced to affect the rate of growth could be determined. At this point, plants in all trays were harvested, divided into various segments, oven-dried, and analyzed.

RESULTS

Critical Nitrogen Concentration in Elodea

The data in Table 2 and the curves in Figure 2 are from an experiment to establish the critical level of nitrogen in the most suitable index segment of Elodea occidentalis. The data on total plant yields in Table 2 show that growth was limited by the nitrogen supply in solutions containing 10.5 and 14.0 ppm nitrogen and reached a maximum of 2.34 g at 21.0 ppm nitrogen. The large increase in yield between 14.0 and 21.0 ppm suggests that the critical concentration is approximately the amount of nitrogen in plants of the 21.0 ppm nitrogen cultures. The nitrogen contents of plants from the 31.5 and 42.0 ppm treatments represent luxury consumption of nitrogen, that is, increasing nitrogen content which did not result in further yield increases.

The second one-inch segment of the main shoots and laterals was selected as the most satisfactory index part for nitrogen. The possible re-export of nitrogen from older to younger tissues under conditions of nitrogen deficiency,

Table 2. The total nitrogen content of the first and second one-inch sections of stems and branches of Elodea occidentalis after culturing in solutions differing in nitrogen content to establish the critical nitrogen concentration in an index segment.

N content of medium (mg/l)	Oven-dry plant wt. (g/2 l)			Tissue N content (%)			
	1	2	Ave.	Plant segment	1	2	Ave.
10.5	1.583	1.575	1.579	1st 1"	1.45	1.49	1.47
				2nd 1"	1.14	1.15	1.14
				3rd 1"	1.07	1.09	1.08
14.0	1.694	1.928	1.833	1st 1"	2.04	1.75	1.89
				2nd 1"	1.52	1.35	1.43
				3rd 1"	1.37	1.09	1.23
21.0	2.428	2.252	2.340	1st 1"	2.13	2.05	2.09
				2nd 1"	1.63	1.55	1.59
				3rd 1"	1.36	1.41	1.39
31.5	1.933	2.371	2.152	1st 1"	4.10	3.21	3.67
				2nd 1"	3.19	2.45	2.82
				3rd 1"	2.54	2.07	2.31
42.0	1.861	1.931	1.896	1st 1"	5.56	5.35	5.45
				2nd 1"	4.38	4.26	4.32
				3rd 1"	3.45	3.31	3.38

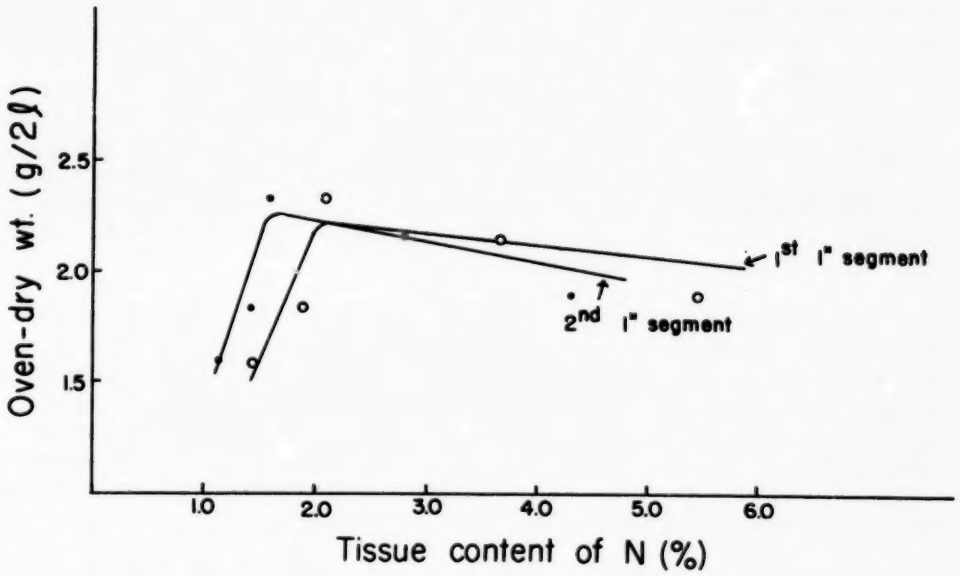

Figure 2. The relationship between yield and total nitrogen content of the first and second one-inch terminal segments of Elodea occidentalis grown in solutions of varying nitrogen content.

and also as a result of senescence and non-nutritional abnormalities in the older tissues made the terminal one-inch segment an unsatisfactory index region. This was supported by unreported results which showed that in plants which had been very deficient in nitrogen for a considerable period, the nitrogen content of terminal one-inch segments was actually above the critical level, apparently due to re-export from extensive regions of older, dying tissues.

A relatively constant concentration of an element throughout the range of yield response to that element is a desirable feature of an index region and was another factor considered in the selection of the second one-inch segment for nitrogen assay. The nitrogen content of the third one-inch segment also varied only slightly (Table 2). However, this segment was rejected as an index region because a large proportion of the lateral brances on Elodea occidentalis were less than three inches in length. In the collection of field samples, it would probably be difficult to obtain sufficient tissue for analysis from the third one-inch segment.

On the basis of the above considerations, the critical nitrogen concentration for Elodea occidentalis was established as 1.60 percent in the second one-inch segment. Yield and nitrogen values for duplicate cultures are included in Table 2 to indicate the variation between replicates in experiments of this type. The nitrogen concentration in the second one inch varied over a considerable range, from 1.14 to 4.32 percent.

The results in Table 3 and Figure 3 are from an experiment to establish the critical concentration of nitrogen for Elodea occidentalis using the tray procedure. In one set of trays, the NO_3-N concentration in the nutrient medium was 42 ppm; in another set, the concentration was only 4.2 ppm. The total nitrogen concentration in the second one-inch segment of nitrogen deficient plants from the low nitrogen trays at harvest was 1.23 percent. This is slightly below the 1.60 percent critical concentration established by the batch procedure. However, as indicated in Figure 3, the Elodea plants probably were deficient in nitrogen several days before a decision to harvest the plants seemed justified. The dry weight of the low nitrogen culture plants was only 393 mg per tray in comparison with 504 mg under high nitrogen. The 14.0 ppm external nitrogen treatment in Table 2 represents a comparable relative yield decrease due to nitrogen deficiency. The nitrogen concentration in the second one-inch segment from that treatment was 1.43 percent which is in reasonable agreement with the 1.23 percent value from the tray experiment.

Table 3. The total nitrogen content of various segments of Elodea occidentalis harvested when nitrogen deficiency had reduced the rate of growth as indicated by length increase.

N content of medium (mg/l)	Plant wt. at harvest (mg)	Tissue content of N (%)		
		1st 1"	2nd 1"	Remainder of plant
4.2	393	1.61	1.23	1.69
42.0	504	5.85	4.34	3.01

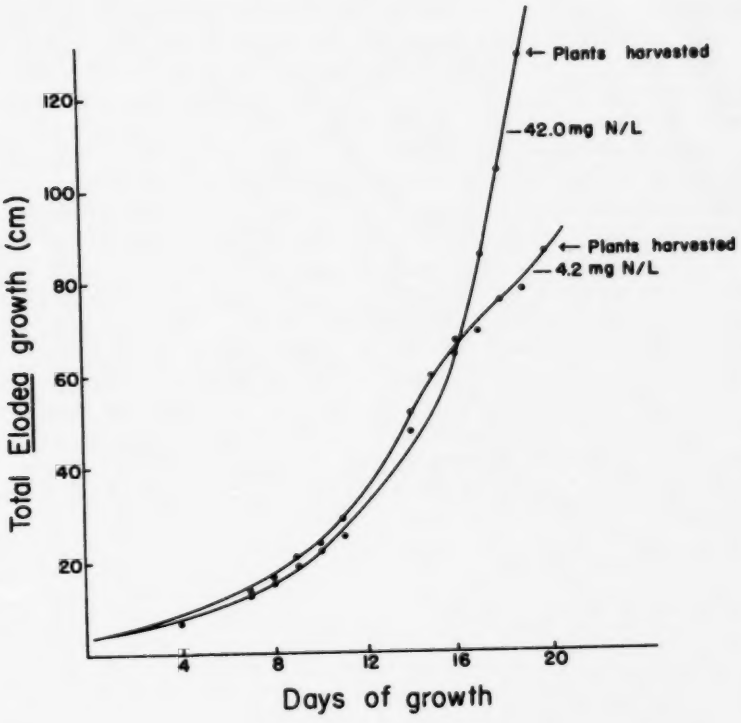

Figure 3. Daily increase in total length of stems and lateral branches of Elodea occidentalis grown in tray cultures under high and low levels of No_3-N.

Critical Phosphorus Concentration in Elodea

Yield and tissue phosphorus concentrations of Elodea grown in nutrient media varying in phosphorus content are presented in Table 4. As with nitrogen, the youngest tissues, that is the terminal one-inch of growth, had the highest phosphorus concentration. This was anticipated because both phosphorus and nitrogen are readily re-exported from younger to older tissues. The second one-inch segment was considered the most satisfactory index segment in which to establish the critical phosphorus concentration.

Dry-weight yields did not level off at a constant maximum as sharply as they did in the nitrogen experiments. Therefore, it was more difficult to establish the critical phosphorus content with certainty. When data of Table 4 were graphed, there was a marked decrease in the yield response per unit of phosphorus made available to the plants at tissue concentrations above approximately 0.14 percent phosphorus in the second one-inch segment. This was considered the critical phosphorus concentration in that segment. The 0.14% value is supported by the data in Table 5 obtained from an experiment in which the critical level of phosphorus was established using the tray procedure. At the time of harvest, the plants were severely phosphorus deficient as indicated by the small amount of growth in the low phosphorus treatment. The phosphorus concentration in the index tissue of these severely deficient plants, 0.12 percent, was slightly lower than the comparable value obtained with the batch procedure. In an earlier study (Gerloff and Krombholz, 1966), the critical phosphorus content for Elodea occidentalis on a whole plant basis was 0.14 percent. It is not surprising that the values based on the entire plant and the second one-inch segment should be the same, because analysis of entire plants represents an average of younger tissues with a higher phosphorus concentration than the second one-inch segment and older tissues with a lower concentration.

Table 4. The total phosphorus content of various portions of Elodea occidentalis after culturing in solutions differing in phosphorus content to establish the critical phosphorus concentration in an index segment.

P content of medium (mg/l)	Ave. oven-dry plant wt.* (g/21)	Plant segment	Tissue P content (%)
0.76	1.383	1st 1"	0.10
		2nd 1"	0.06
		3rd 1"	0.06
		Remainder	0.09
1.16	1.730	1st 1"	0.14
		2nd 1"	0.08
		3rd 1"	0.07
		Remainder	0.10
1.55	1.984	1st 1"	0.16
		2nd 1"	0.10
		3rd 1"	0.11
		Remainder	0.11
3.1	2.329	1st 1"	0.25
		2nd 1"	0.18
		3rd 1"	0.17
		Remainder	0.17
6.2	2.788	1st 1"	0.39
		2nd 1"	0.35
		3rd 1"	0.33
		Remainder	0.31

* Averages of duplicate cultures.

Table 5. The total phosphorus content of various segments of Elodea occidentalis harvested when phosphorus deficiency had reduced the rate of growth as indicated by length increase.

P content of medium (mg/l)	Plant wt. at harvest (mg)	Tissue content of P (%)			
		1st 1"	2nd 1"	3rd 1"	Remainder of plant
0.2	142	0.12	0.12	0.09	*
6.2	625	0.95	0.75	0.71	0.78

* Insufficient tissue for analysis

Critical Concentrations of Additional Elements

The results of experiments to establish the critical concentrations of elements other than nitrogen and phosphorus in index segments of Elodea are summarized in Table 6. As anticipated, the critical concentrations for the trace elements were much lower than for the major essential elements. Values ranged from 8 ppm for zinc to only 0.15 ppm for molybdenum. The total range of concentration of each element also is indicated in Table 6. It is apparent that the concentrations of the essential elements in Elodea are not fixed values but vary over a considerable range.

Critical concentrations were not established for the trace elements chlorine and copper. Because of the low plant requirements for chlorine and the large quantities in rainfall as a result of atmospheric contamination, it is unlikely chlorine would be a limiting factor in macrophyte growth under field conditions. This is not true of copper. As established in Section V of this report, there is a strong possibility that copper supply is growth limiting in some northern Wisconsin lakes. Unfortunately, in spite of elaborate purification efforts, several experiments to establish a critical copper concentration in Elodea were unsuccessful.

Table 6. Critical concentrations and the range of concentrations of essential nutrient elements in index segments of Elodea occidentalis.

Element	Index segment	Critical conc.	Range in conc.
N	2nd 1"	1.60%	1.14- 4.32%
P	2nd 1"	0.14%	0.06-0.35%
S	2nd 1"	0.08%	0.06-0.46%
Ca	1st 1"	0.28%	0.17-0.62%
Mg	2nd 1"	0.10%	0.06-0.19%
K	2nd 1"	0.80%	0.25-2.51%
Fe	1st 1"	60 ppm	40-219 ppm
Mn	1st 1"	4.0 ppm	2.2 -16.7 ppm
Zn	2nd 1"	8.0 ppm	3.6 -34.4 ppm
Mo	1st 1"	0.15 ppm	0.04-6.4 ppm
B	1st 1"	1.3 ppm	0.3 -11.2 ppm

Critical Concentrations in Ceratophyllum

Although Elodea occidentalis was selected as the primary organism on which to base the plant analysis assay, the critical concentrations of nitrogen, phosphorus, and several other elements were established in Ceratophyllum demursum. These are presented in Table 7.

The critical nitrogen concentration in the second one-inch segment of Ceratophyllum was 1.30 percent. This value is in agreement with the critical concentration reported for entire Ceratophyllum demursum plants in a previous

Table 7. Critical concentrations and the range of concentrations of several essential nutrient elements in index segments of Ceratophyllum demursum.

Element	Index segment	Critical conc.	Range in conc.
N	2nd 1"	1.30 %	1.00 - 2.42 %
P	2nd 1"	0.10 %	0.09 - 0.41 %
Ca	1st 1"	0.22 %	0.11 - 0.47 %
Mg	2nd 1"	0.18 %	0.11 - 0.40 %
K	2nd 1"	1.70 %	1.58 - 5.10 %
B	1st 1"	2.8 ppm	1.49 - 12.54ppm

study (Gerloff and Krombolz, 1966). The critical phosphorus concentration in the second one-inch was 0.10 percent. A comparison of Tables 5 and 7 shows that the critical nitrogen and phosphorus concentrations were somewhat lower in Ceratophyllum than in Elodea. This probably is related to a higher stem to leaf ratio in Ceratophyllum.

The greatest difference in critical concentrations in the two species was in potassium. The critical potassium concentration in Elodea was only 0.80%; in Ceratophyllum, it was 1.70% or slightly more than double the Elodea value. Only further experimentation can determine if this difference is significant in terms of growth and distribution of the plants in lakes and streams.

DISCUSSION

Two aspects of this study seem critical in developing plant analysis as a practical nutrient assay for aquatic environments. First, critical concentrations were established in Elodea occidentalis for most of the essential elements rather than just nitrogen and phosphorus. This greatly increases the usefulness of the technique. It also has made this initial effort with aquatic environments one of the most complete studies of plant analysis with any species. Secondly, it is considered important that the technique has been based on critical concentrations in index segments. This should minimize errors resulting from sampling either terminal tissues, which might be disproportionately high in some elements even under deficiency conditions, or older senescent tissues, which could have a low content of the elements even under adequate nutrient supplies.

Interpretation of the plant yield-tissue content response curves is subjective to some degree. The curves often do not agree with the theoretically ideal curve (Ulrich, 1952). There has also been some disagreement among investigators on the point on the response curve at which the critical level should be established. The approach in this study was to consider the critical concentration the tissue content at a point approximately 5 percent below the maximum yield represented by the leveling off of the response curve. This conforms with the interpretation of numerous investigators when applying the tissue analysis technique to the culture of agricultural and horticultural crops.

The reproducibility and reliability of the reported critical contents are of some concern. The degree of variation in yield and in tissue content of nitrogen in duplicate cultures of the same experiment was indicated in Table 2. This is typical of the best experiments in this study. Better agreement in replicated cultures cannot be expected as long as the plants are propagated vegetatively. Even with the most careful selection of plant segments when subculturing, it is impossible to obtain uniform initiation of growth from terminal and lateral buds.

The results from this and a related study (Gerloff and Krombholz, 1966) allow comparison of critical values for the same element established in separate experiments and by different techniques. In a particular species it

seems possible to reproduce the nitrogen critical level to ± 0.1 percent and the phosphorus level to ± 0.01 percent. This suggests the desirability of reporting the critical percentage as a range rather than a specific value. However, the common practice in agricultural applications has been to report specific values. That practice has been continued here. The degree of reliability indicated above must be recognized and understood in using the critical concentrations. The values reported here seem fully as reliable as those reported for crop species.

SECTION V

EVALUATION OF PLANT ANALYSIS FOR THE ASSAY OF NUTRIENT SUPPLIES IN WISCONSIN LAKES

After critical concentrations of the essential elements in Elodea occidentalis and Ceratophyllum demursum were established in laboratory experiments, plant analysis was used to evaluate nutrient supplies and growth-limiting nutrients in Wisconsin lakes. This involved collecting samples of the first and second one-inch segments of the Elodea and Ceratophyllum from lakes known to vary considerably in fertility. Samples were obtained at intervals throughout the growing season, were analyzed for the essential elements, and the values were compared with the critical concentrations. Concentrations of an element consistently above the critical level were interpreted to indicate the element was in abundant supply and not limiting plant growth; a concentration at or below the critical level indicated supplies of that element had become limiting at the time of sampling. In this case, restriction of entry of the growth-limiting element into the specific body of water could be expected to reduce growths of nuisance macrophytes. Conversely, additions of the element probably would increase macrophyte growth.

EXPERIMENTAL PROCEDURE

During the summer of 1970, samples of Elodea occidentalis, the primary assay organism, were routinely collected from 9 Wisconsin lakes. All of the lakes sampled except one are located in or near Vilas County in northern Wisconsin in non-agricultural areas. They are relatively infertile. Water hardness varied from 14 to 65; the pH of the water was in the range of 7.1 to 8.0 (Black, Andrews, and Threinen, 1963).

Plant samples collected from lakes were lifted from the water with a garden rake and taken to the laboratory while immersed in lake water. Within several hours, sufficient first and second inch segments to represent 3-4 g of oven-dry material were cut from the main branches and laterals of healthy, green plants. After repeated rinsing in lake water to eliminate debris, the samples were soaked 30-45 seconds in 0.2N HCl, rinsed with lake water,

rinsed in distilled water, placed in nylon bags, and dried to constant weight at 65-70°C.

Total nitrogen analyses on all field samples were by a semimicro Kjeldahl procedure. In addition, all samples were analyzed for 10 elements by the Jarrell-Ash Multichannel Emission Spectrometer at the Wisconsin Alumni Research Foundation Laboratories. In all lakes in which there were indications from the emission spectrometer analyses that a specific element had become limiting, or was close to limiting, for plant growth, analyses were verified by the quantitative procedures used in establishing critical concentrations in the laboratory. This included analyses for calcium, phosphorus, and copper.

Samples analyzed by the Jarrell-Ash Spectrometer were ground in a Wiley Mill equipped with a stainless steel screen. Samples analyzed by conventional quantitative procedures were ground with an agate mortar and pestle to minimize trace element contamination.

Some additional samples were obtained from natural macrophyte populations in Wisconsin lakes during the summer of 1971. However, emphasis was shifted to another use of the plant analysis technique. The Elodea bioassay organism was placed in porous, inert containers near the surface in lakes in which nutrient supplies were to be evaluated. Samples of the first and second one-inch index segments were to be removed periodically, analyzed for elements suspected to limit growth, and evaluated by comparisons with the critical concentrations. The anticipated advantage of this procedure was that the assays would primarily reflect nutrient availability in the water layer and would be relatively uninfluenced by the direct abosrption of nutrients from sediments.

Cylindrical baskets 8-1/2 inches in diameter and 12 inches long were constructed of 1/4 inch mesh polyethylene screen. The cylinders were closed at the ends with the polyethylene mesh and were reinforced with plastic rings 1/4 inch wide. After the baskets were anchored in relatively sheltered areas of lakes in the vicinity of macrophyte beds where the water was no more than 5 feet deep, an inoculum of laboratory-grown Elodea or Ceratophyllum was placed in each. The baskets were floated near the water surface by two empty, sealed one-gallon polyethylene bottles. One basket of each of the two macrophytes was placed in 5 northern Wisconsin lakes.

RESULTS

Because of the large amount of data, only analyses of the macrophytes from representative lakes are presented and discussed in this section. Complete analytical data on the samples from the additional lakes are presented in the Appendix.

Samples of 1970

The analyses of Elodea from one of the most fertile northern Wisconsin lakes sampled (Little John) and one of the least fertile (Salsich) are presented in Tables 8 and 9.

Concentrations of nitrogen and phosphorus in the lake plants are of particular interest because these elements most frequently have been considered limiting for aquatic plant growth. In all Elodea samples from Little John Lake, the nitrogen and phosphorus concentrations were well above the critical concentrations of 1.60% and 0.14% in the second one-inch segment. The lowest phosphorus concentration was 0.40% on July 27 indicating very adequate supplies of that element all during the growing season.

The lowest concentrations of elements in general were observed in samples from July 27. This sampling apparently was in the period of most rapid plant growth and greatest pressure on nutrient supplies. However, even in the July 27 samples, the concentrations of all elements, except nitrogen were at least double the critical concentrations reported in Table 6.

The nitrogen and phosphorus analyses of Elodea samples from Salsich Lake contrast with the results from Little John. The nitrogen concentrations were in general slightly higher in the Salsich samples, but the phosphorus concentrations were considerably lower. For example, in the second one-inch segment from July 28 the phosphorus concentration was only 0.18% and in the August 19 sample 0.15%. Comparable phosphorus values in the Little John samples were 0.40 and 0.54%. The low values for Lake Salsich plants, which were verified with a standard colorimetric procedure, approach the critical concentration of 0.14%, and indicate that in this lake phosphorus supplies were close to limiting for Elodea growth. Further additions of phosphorus could be expected to increase the

Table 8. Essential element content of first and second one-inch segments of Elodea occidentalis collected from Little John Lake during the summer of 1970.

Date Sampled	Segment	%					Ppm				
		N	P	K	Ca	Mg	Fe	B	Cu	Mn	Zn
June 16	1"	3.50	0.65	2.30	0.52	0.22	>1000	21.1	1.3	404	46
	2"	3.35	0.70	2.30	0.85	0.23	>1000	24.9	2.1	>410	35
July 6	1"	3.05	0.54	2.75	0.54	0.22	795	13.6	1.5	>410	71
	2"	2.66	0.54	2.55	0.91	0.19	>1000	14.9	2.6	>410	86
July 27	1"	2.81	0.47	2.45	0.68	0.21	665	13.5	2.2	386	56
	2"	2.22	0.40	2.45	1.07	0.19	855	16.6	<1.0	>410	74
Aug. 18	1"	3.58	0.65	2.80	1.09	0.25	905	14.9	3.4	254	95
	2"	2.84	0.54	3.25	1.55	0.20	820	13.3	1.8	>410	89
Sep. 8	1"	3.49	0.80	2.55	1.09	0.24	760	13.5	3.6	360	66
	2"	3.10	0.68	3.45	1.17	0.19	625	12.5	<1.0	>410	81

Except for N, all analyses were made with a Jarrell-Ash direct-reading, computer-programmed emission spectrometer.

Table 9. Essential element content of first and second one-inch segments of Elodea occidentalis collected from Salsich Lake during the summer of 1970.

Date sampled	Segment	%					Ppm				
		N	P	K	Ca	Mg	Fe	B	Cu	Zn	Mn
July 7	1"	3.78	0.35	2.20	0.48	0.21	555	12.1	6.2	197	98
	2"	3.47	0.21	2.80	0.64	0.17	445	13.1	4.3	182	150
July 28	1"	3.55	0.25	2.20	0.42	0.19	450	12.4	5.0	160	116
	2"	3.14	0.18	2.65	0.66	0.16	480	13.5	3.8	224	190
Aug. 19	1"	3.50	0.33	2.65	0.50	0.20	545	11.5	4.7	162	124
	2"	3.02	0.15	2.80	0.62	0.16	430	8.8	1.8	156	185
Sep. 10	1"	3.47	0.40	2.35	0.64	0.18	485	8.4	3.1	177	137
	2"	2.83	0.40	2.10	0.91	0.19	580	12.5	4.0	177	225

Except for N, all analyses were made with a Jarrell-Ash direct-reading, computer-programmed emission spectrometer.

presently sparse weed beds in Salsich Lake.

Because of inadequate plant tissue, it was impossible to run molybdenum analyses on all samples from the 9 lakes. The range of molybdenum in 7 samples analyzed was 0.50 to 0.73 ppm, in the terminal one-inch, with an average of 0.57 ppm. Even the lowest concentration was 3 to 4x the critical concentration of 0.15 ppm presented in Table 6, thus eliminating the need to consider molybdenum as a possible growth-limiting factor.

The copper analyses reported in Tables 8 and 9 are of interest because some are sufficiently low to suggest Cu deficiency. This cannot be verified until the copper critical concentration is firmly established in laboratory experiments. However, comparisons with critical concentrations determined for agricultural and horticultural terrestrial species support the indicated copper deficiency. For example, a critical copper concentration of 5 ppm has been reported for corn, alfalfa, and soybeans and 7 ppm for alfalfa (Melstead, Motto, and Peck, 1969). Less than 4 ppm Cu in the leaves was associated with copper deficiency in citrus (Chapman, 1961). Several of the copper concentrations in Elodea from Salsich and all of the values from Little John were less than 4 ppm. For a number of samples the copper data reported in Tables 8 and 9 were checked by atomic absorption analysis. The atomic absorption values were consistently higher. However, the copper contents obtained by atomic absorption still were below the 4-7 ppm critical concentrations for economic crops and confirm that either Elodea was copper deficient in Little John Lake, or that it has an unusually low requirement for that element.

Although the critical copper concentration has not as yet been determined, it has been possible to grow Elodea severly deficient in copper. Yields from cultures to which copper was not added were only 25 percent (0.909 g) of yields (3.636 g) with copper added. The copper concentration in the first two inches of the copper deficient plants was 1.57 ppm; in normal plants, it was 17.7 ppm. From experience with the other trace element cations, it can be anticipated that the critical copper concentration will be at least double the 1.57 ppm in the deficient plants. This supports the view that copper supplies did become growth-limiting in some of the lakes sampled.

In Table 10, data are presented for samples taken from 8 lakes during the late July period of maximum stress on nutrient supplies. Analyses are reported only for the four elements which critical concentration comparisons indicated might limit plant growth. When the Jarrell-Ash data were verified by standard quantitative procedures, data obtained by both types of analyses are presented.

Comparisons with analyses of samples from other lakes emphasize that the phosphorus concentration in plants from Salsich Lake (0.18%) was relatively low. Whitney Lake also seems close to phosphorus deficiency for Elodea growth, as indicated by the 0.17% value for the index segment. The data on Clear Lake are of interest because the phosphorus concentration was relatively high at 0.24% but the nitrogen concentration of 1.94% in the second one-inch was the lowest of any sample, and only slightly above the 1.60% critical value. The 2.70% and 0.29% concentrations of nitrogen and phosphorus, respectively, in the Erickson Lake samples were high, but the 0.28% calcium concentration in the terminal one-inch index segment was at the critical level. It is not surprising that calcium might be a limiting factor in soft water lakes and that species with a higher requirement for calcium than Elodea, or less capacity to absorb calcium from the environment, might be eliminated from soft water lakes such as Erickson.

Probably the most consistently low values in Table 10 are to be observed in the copper data. In Elodea from 5 of the 8 lakes copper concentrations were low enough to suggest a growth-limiting role of that element.

In contrast to the other 6 lakes, Allequash and Little Spider Lakes seem relatively well supplied with all the essential nutrient elements.

The data in Table 11 are from analyses of Ceratophyllum demursum index segments from Lake Mendota at Madison, Wisconsin. Mendota is an extremely fertile, hard-water lake which has extensive beds of macrophytes and troublesome algae blooms. The unusual fertility of Lake Mendota is reflected in the very high concentrations of nutrient elements in the samples. For example, the average concentration of nitrogen in the second one-inch segment was 3.75%; the phosphorus concentration was 0.65%. These values are well above comparable values in plants from the northern Wisconsin lake and are approximately 3x and 6x

Table 10. Concentrations of potentially growth-limiting elements in Elodea occidentalis collected from northern Wisconsin lakes during late July, 1971.

Lake sampled	Date sampled	Methyl orange alk.*	Plant segment	Concentration of N	P		Ca		Cu
					JAS**	Std.**	JAS	JAS	Std.
Allequash	July 27	39	1"	3.53	0.34	0.31	0.42	3.8	6.5
			2"	3.19	0.28	0.22	0.61	3.4	7.0
Clear	July 29	33	1"	2.41	0.42	0.34	0.69	2.2	
			2"	1.94	0.28	0.24	0.66	1.8	
Whitney	July 29	22	1"	2.34	0.23	0.21	0.68	2.2	
			2"	2.02	0.21	0.17	0.89	1.8	
Erickson	July 28	24	1"	3.41	0.47	0.41	0.28	3.8	
			2"	2.70	0.29	0.28	0.44	2.2	
Big Kitten	July 29	65	1"	2.71	0.42	0.39	1.61	<1.0	3.9
			2"	2.16	0.40	0.34	1.51	2.2	2.7
Salsich	July 28	14	1"	3.55	0.25	0.26	0.42	5.0	
			2"	3.14	0.18	0.18	0.66	3.8	
Little John	July 27	45	1"	2.81	0.47	0.35	0.68	2.2	2.4
			2"	2.22	0.40	0.25	1.07	<1.0	3.3
Little Spider	July 28	--	1"	3.36	0.44	0.37	0.52	3.8	6.5
			2"	2.59	0.28	0.27	0.61	2.2	4.5

* Values for methyl orange alkalinity were reported by the Wisconsin Department of Natural Resources.

** JAS indicates analyses made with a Jarrell-Ash direct-reading, computer-programmed emission spectrometer; Std. refers to analyses by standard colorimetric, emission flame photometer, and atomic absorption procedures.

Table 11. Essential element content of first and second one-inch segments of Ceratophyllum demursum collected from Lake Mendota during the summer of 1970.

Date sampled	Segment	%						Ppm					
		N	P	S	K	Ca	Mg	Fe	B	Cu	Zn	Mn	Mo
June 18	1"	3.75	0.49		4.25	0.48	0.81	460	15.4	3.9	101	>1000	0.42
	2"	2.90	0.39		3.45	0.52	0.98	540	19.5	3.7	121	>1000	
July 20	1"	5.40	0.92		4.45	0.45	0.69	920	16.6	12.3	163	>1000	
	2"	4.35	0.80		4.25	0.47	0.68	890	18.8	9.8	203	>1000	
Aug. 8	1"	4.28	0.70		4.80	0.31	0.70	495	12.7	12.8	70	247	
	2"	4.21	0.61		4.70	0.55	0.69	565	13.3	7.6	70	>1000	
Sept. 1	1"	4.14	0.77	0.32	3.10	0.50	0.88	>999	16.4	14.2	117	388	
	2"	3.55	0.80	0.40	3.95	0.71	0.89	930	16.8	10.8	131	>1000	

Except for N, all analyses were made with a Jarrell-Ash direct-reading, computer-programmed emission spectrometer.

the critical concentrations for nitrogen and phosphorus, respectively. Concentrations of all the trace elements also were relatively high. The data suggest it would be necessary to remove considerable quantities of nitrogen, phosphorus, or any other essential nutrient, from pollution sources entering Lake Mendota to significantly reduce nuisance macrophyte growths.

Samples of 1971

In Table 12, data are presented from the analyses of Elodea and Ceratophyllum which had been in the assay baskets for approximately two months, from late June until early September. Samples also were collected in late July from the natural populations in these lakes and analyzed. Unfortunately, both species were not present in all 5 lakes. The concentrations of nitrogen and phosphorus were well above the critical concentrations of 1.60% nitrogen and 0.14% phosphorus in Elodea and 1.30% and 0.10% in Ceratophyllum in all samples from the natural populations. There was no indication either nitrogen or phosphorus was growth limiting in early July.

Comparisons of nitrogen and phosphorus concentrations in natural populations of the two species obtained from the same lake (Clear or Little John) are of interest. Elodea produces roots which presumably are imbedded in bottom sediments while Ceratophyllum has almost no roots. Elodea, therefore, obtains nutrients from both the mud and water; Ceratophyllum must rely on the water. Nitrogen concentrations were much lower in Ceratophyllum than in Elodea in both Clear and Little John lakes suggesting the advantage of roots in drawing on bottom sediment nitrogen supplies. There was little difference in the phosphorus concentrations in the two species, indicating an unusual capacity of Ceratophyllum to absorb phosphorus from the very low concentrations in the water. The collections of 1970 indicated Allequash to be a fertile lake. This correlates with the lack of a difference in nitrogen concentrations in Elodea and Ceratophyllum from this lake.

Unfortunately, the Elodea and Ceratophyllum inoculated into the baskets grew very poorly. There was hardly enough tissue for nitrogen and phosphorus analyses by early September when the baskets were removed from the lakes. Whether this was due to the shock of transfer of laboratory-grown plants to the lake environments, to placement of the plants too near the surface, or to other

Table 12. Nitrogen and phosphorus analyses of index segments of Elodea occidentalis and Ceratophyllum demursum from natural populations and assay baskets in northern Wisconsin lakes.

Lake	Natural pop's. 7-31-71				Assay baskets 9-11-71			
	Elodea (%)		Ceratophyllum(%)		Elodea (%)		Ceratophyllum(%)	
	N	P	N	P	N	P	N	P
Nebish	4.11	0.32	--	--	1.20	0.08	1.90	0.24
Clear	2.68	0.37	1.69	0.28	1.58	0.11	1.64	0.19
Allequash	2.65	0.21	3.00	0.24	1.57	0.26	--	--
Little John	2.30	0.27	1.73	0.31	1.04	0.12	1.83	0.24
Salsich	--	--	--	--	1.25	0.09	1.50	0.15

reasons must be determined in future studies. The low concentrations of both nitrogen and phosphorus in the Elodea suggests this was due to poor physiological condition induced by factors other than nutrient deficiencies. In the Ceratophyllum samples, nitrogen was proportionally closer to the critical concentration (1.30%) than was phosphorus (0.10%).

The similarity in nitrogen and phosphorus concentrations in the Ceratophyllum from the natural populations and the baskets suggests the absence of roots might make Ceratophyllum a more suitable assay organism than Elodea for evaluating nutrient supplies for all non-rooted green plants including algae.

DISCUSSION

The results obtained suggest plant analysis can be a useful relatively simple procedure for nutrient assay in aquatic environments, and that it is a procedure which offers several advantages. Plant analysis minimizes the difficulties associated with obtaining representative samples of the aquatic environment. In this procedure, plants become the sampling device, and a single analytical value provides an integrated expression of all the factors which affected the availability of an element in the micro-environments to which the plants were exposed during growth. Chemical analyses of water samples must be interpreted in terms of fractions available and unavailable to plants and concentrations and quantities which become limiting for growth. These problems are reduced in the plant analysis procedure, because plant analysis is based on values which have been correlated with plant growth and yield responses.

Two aspects of the field data seem worthy of comment. First, although nitrogen and phosphorus are the elements of most interest in practical pollution control, the data indicated that neither element was a general limiting nutrient for Elodea in the lakes sampled. In some lakes, nitrogen or phosphorus was near the critical concentration. In these ecosystems, reduction in available supplies of nitrogen or phosphorus, whichever was limiting, probably, would reduce macrophyte populations; also, further eutrophication with these elements would accentuate nuisance growths. A second point of interest, and

obviously in need of verification, is the suggested copper deficiency in northern Wisconsin lakes. This focuses attention on the importance of trace elements in the field nutrition of aquatic plants. Copper deficiency also would be of some practical importance, because if that element controls plant growth in certain lakes, limited reductions in nitrogen or phosphorus pollution might not improve nuisance conditions in those lakes to the degree anticipated.

Although the results presented suggest plant analysis is a useful bioassay, the need for additional studies to refine the technique is emphasized. Hundreds of investigations have provided the information necessary to firmly establish plant analysis as a procedure for nutrient assays in soils. Undoubtedly, there will be unique aspects in applications of plant analysis to aquatic plants and environments. Further investigations of the most suitable index segments, more precise establishment of critical concentrations, and the determination of critical concentrations for other macrophytes and algae seem worthy of immediate study.

SECTION VI

OPTIMUM NUTRIENT SOLUTION AND GROWTH CONDITIONS FOR THE LABORATORY CULTURE OF NUISANCE MACROPHYTES

Successful laboratory studies of the nutrition and physiology of any plant are facilitated by the availability of a suitable nutrient culture medium and knowledge of optimum growth conditions. A satisfactory culture medium is one in which essential elements do not become growth limiting during extended culture periods, total salt concentration and concentrations of heavy metals are below toxic levels, and the pH remains within a suitable range as plants absorb nutrients from the medium.

Several procedures have been used in formulating nutrient media, including approximation of proportions of the essential elements in soil extracts, determining the amounts of elements taken up from nutrient solutions, and by measuring responses to systematic variations in the concentrations of individual elements in a medium.

Relatively little laboratory work has been carried out in which macrophytes have been cultured in synthetic media (Bourn, 1932). In studies prior to this project, it had been established that macrophytes made reasonably satisfactory growth in a modified Hoaglands solution (Gerloff and Krombholz, 1966), a medium used for many years in laboratory and greenhouse studies with crop plants. The modified Hoagland's medium was used in the studies presented in Section IV of this report. Systematic variations of the concentrations of each essential element in these experiments made it possible to estimate the minimum concentration of each element in a culture medium for optimum growth of the macrophytes under study. The formulation of a culture medium based on these optimum concentrations was suggested. In Section VI experiments will be discussed in which this medium was systematically varied to develop a general macrophyte nutrient culture solution. Results also are presented from several studies to determine optimum conditions of temperature and light for macrophytes.

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EXPERIMENTAL PROCEDURES

The general culture conditions and harvesting procedures employed were described in Section IV.

Several chelates of iron were compared as an iron source, primarily EDTA (ethylenedinitrilo)-tetraacetic acid and EDDHA ethylenediamine di-(o-hydroxyphenylacetate) The EDDHA was obtained from Geigy Chemical Co. as an 86.3 percent pure salt. Iron chelates were prepared as a 0.01 M stock solution of FeEDTA (2.78 g $FeSO_4 \cdot 7H_2O$ and 3.72 g Na_2EDTA) and as a 0.01 M stock solution of FeEDDHA (140 mg KOH, 278 mg $FeSO_4 \cdot 7H_2O$, and 424 mg EDDHA).

RESULTS

The composition of the macrophyte culture medium developed on this project is presented in Table 13.

Concentrations of Elements

The initial step in formulating the macrophyte medium was to determine from the critical concentration experiments in Section IV the minimum concentration of each essential element which was just adequate to produce maximum growth of Elodea in a 4-week period in two liters of solution. These values were compared with the amounts of the elements necessary to maintain the critical concentration in 5.0 g of oven-dry growth of Elodea and Ceratophyllum. From these comparisons some upward adjustments in element concentrations seemed necessary, particularly in the three major element cations, calcium, magnesium, and potassium. As a result, the concentrations of the essential elements in the solution of Table 13 are approximately double the minimum concentrations for maximum yield derived from critical concentration experiments.

Providing each element at the lowest but adequate concentration was considered desirable to reduce the possibility of adverse effects on growth due to high salt concentrations and toxic trace element concentrations. Minimum deviation from the low concentrations characteristic of lakes and streams also seemed desirable.

Table 13. Composition of medium recommended for macrophyte culture

Salt	Stock sol'n, wt./liter	Ml stock/liter final solution	Element in final solution, ppm
KNO_3	10.10 g	5	N - 39.9
$Ca(NO_3)_2 \cdot 4H_2O$	37.76 g	5	K - 27.3
$MgSO_4 \cdot 7H_2O$	14.79 g	5	Ca - 32.0
KH_2PO_4	5.44 g	5	Mg - 7.2
$NaNO_3$	12.75 g	5	S - 9.6
			P - 6.2
KCl	746 mg	1	Cl - 0.354
H_3BO_3	155 mg	1	B - 0.027
$MnSO_4 \cdot H_2O$	169 mg	1	Mn - 0.054
$ZnSO_4 \cdot 7H_2O$	115 mg	1	Zn - 0.026
$CuSO_4 \cdot 5H_2O$	12.5 mg	1	Cu - 0.003
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	3.7 mg	1	Mo - 0.002
*FeEDDHA	---	10	Fe - 0.56

* FeEDDHA solution is prepared by dissolving 140 mg KOH in 400 ml double distilled water, adding 424 mg 85% pure EDDHA, and stirring until dissolved. Finally 278 mg $FeSO_4 \cdot 7H_2O$ dissolved in 500 ml double distilled water is added and the solution is diluted to 1 liter.

Chemical Salts

Solubility and effect on the pH of the medium throughout the growth period are primary factors in the selection of salts to provide essential elements in a nutrient medium. The four salts $Ca(NO_3)_2 \cdot 4H_2O$, KNO_3, $MgSO_4 \cdot 7H_2O$, and KH_2PO_4 have been widely used in plant culture media, including the modified Hoagland's solution from which the macrophyte medium was derived. The $NaNO_3$ in the recommended medium is to provide additional nitrogen without increasing the concentration of another essential element.

As will be apparent in data to be presented, the pH of the macrophyte solution immediately after preparation and autoclaving was approximately 5.0, and after equilibrium with the 1% CO_2 in air mixture it was 4.9. As plants absorbed ions, the pH of the medium increased so that at harvest it correlated to a degree with plant yields. In general, pH values at harvest were in the range of 6.0 to 7.5 for Elodea and Ceratophyllum and somewhat higher for Myriophyllum, as high as 10.0. Differences in yield and absorption of ions account for these variations.

Trace Element Concentrations

The primary difference in the culture medium used in the critical concentration experiments of Section IV and the medium derived from those experiments is the concentrations of the trace elements which were reduced to 1/5 to 1/10 the concentrations in the original medium. This seems desirable because, except for chlorine, the range in concentration between adequacy and toxicity of the trace elements can be narrow as illustrated in the data of Table 14 showing the response of Elodea to copper. The average yield from triplicate cultures provided with 0.006 ppm copper was 1.352 g; with 0.03 ppm copper, yield was reduced to 1.098 g; and with 0.15 ppm, yield was only 0.531 g. The adverse effects of concentrations of trace elements close to the toxicity level probably are primarily on the inoculum and on the initiation of growth in a culture. If the concentration of an element is not so high that the inoculum is killed, the amount of the element per unit of material is reduced as growth develops and initial toxicity effects in a culture no longer will be evident.

Table 14. Toxicity of copper to Elodea occidentalis.

Cu in culture soln., ppm	Yield of Elodea, g/21.*
0.0	1.325
0.003	1.399
0.006	1.352
0.03	1.098
0.15	0.531

* Average of triplicate cultures.

Iron Source

Maintenance of adequate available iron is a critical factor in successful growth of plants in aerated, liquid, culture media. The low solubilities of iron precipitates which form at the slightly acid and alkaline pH values of nutrient media make inorganic iron sources, in general, unsatisfactory. As a result, chelating agents have been employed for a number of years to maintain iron availability. Ferric citrate and particularly FeEDTA have been effective in many culture media. Because of a capacity to maintain iron in a soluble form at higher pH values than does EDTA, EDDHA has recently been the iron source in several culture media (Wallace, 1966).

The importance of the iron source in the culture of macrophytes is indicated in Table 15. Ferric citrate was completely unsatisfactory as a source of iron for Ceratophyllum demursum. FeEDTA was far superior to ferric citrate but was less satisfactory than FeEDDHA. Because of the increase in the pH of the culture medium associated with autoclaving, it seemed desirable to autoclave the iron source separately and add it to culture media after autoclaving. However, the data indicate separate autoclaving was not beneficial.

Table 15. Comparison of several iron sources for the growth of Ceratophyllum demursum in a synthetic culture medium (0.56 ppm Fe provided from each Fe source).

Fe source	Oven-dry plant wt (g/l)*	pH of medium at harvest
Fe citrate, autoclaved separately	0.008	5.72
FeEDTA, autoclaved separately	0.833	7.00
FeEDTA, autoclaved with the medium	0.843	6.95
FeEDDHA, autoclaved separately	1.315	7.14
FeEDDHA, autoclaved with the medium	1.373	7.10

* Each value is an average of results from duplicate cultures.

From experience on this project, it is difficult to generalize on the response of macrophytes to a specific concentration or source of iron. Even the addition of 0.56 ppm iron as FeEDDHA has not always provided adequate iron. Also, FeEDTA sometimes is as suitable an iron source as is the EDDHA complex. The most satisfactory results have been obtained by adding 0.56 ppm iron as FeEDDHA when the culture medium is prepared and then a second and often a third increment of 0.28 ppm iron after growth is initiated. The supplemental iron is added aseptically when the red color of the FeEDDHA complex has nearly disappeared, usually 7 to 10 days into the culture period. A third addition may be necessary if the red color again disappears.

Yield Comparisons

As indicated in Table 16, evaluations of the recommended macrophyte medium were possible through comparisons of plant yields in that medium, in modifications of it, and in the original medium.

For Elodea, the recommended medium (x macrophyte) produced as much yield as the original solution in two experiments. Dilution of the recommended medium (x/2 macrophyte) reduced the higher yield. Apparently the supply of one or several elements had become limiting. Doubling the concentrations of all constituents (2x macrophyte) reduced yield slightly.

The proposed macrophyte solution was also equal to, or somewhat superior to, the original medium in the culture of Myriophyllum. Dilution to half-strength did not reduce yields with Myriophyllum, probably because its requirements for nitrogen and phosphorus are less than the requirements of Elodea.

The recommended medium has not been quite as effective for the culture of Ceratophyllum as was the original medium. This is verified by data from two experiments reported in Table 16. The reason is unclear. Ceratophyllum does have a higher potassium critical concentration than Elodea. However, increased solution concentrations of potassium did not increase yields. Higher concentrations of individual trace elements also were without effect. Ceratophyllum demursum in general has been a less satisfactory experimental organism than Elodea occidentalis or Myriophyllum spicatum, primarily because of sudden leaf drop from the stems and branches after several weeks of culture. This accounts for the relatively low yields of Ceratophyllum in Table 16.

Probably the most unexpected result in Table 16 was the positive response of Elodea and Myriophyllum to the addition of Na_2CO_3 (120 mg per liter) to the medium. The effect was particularly evident as more rapid growth in the early stages of the cultures. It is understandable, therefore, that the response of various species to Na_2CO_3 and of the same species in different experiments might vary. Macrophyte yields were not increased by the addition of $Na_2SiO_3 \cdot 5H_2O$. This suggests the beneficial effect of Na_2CO_3 was on carbonate-bicarbonate ratios in the medium rather than on pH.

Table 16. Comparisons of the growth of three macrophyte species in an initial culture medium and in modifications of a newly developed medium.

Culture medium	*Elodea occidentalis*				*Myriophyllum spicatum*				*Ceratophyllum demursum*			
	Exp. 1		Exp. 2		Exp. 1		Exp. 2		Exp. 1		Exp. 2	
	Yield (g/21)	Final pH	Yield (g/21)	Final pH	Yield (g/21)	Final pH	Yield (g/21)	Final pH	Yield (g/21)	Final pH	Yield (g/21)	Final pH
Original	2.732	6.81	3.251	--	3.514	9.73	4.594	10.0	1.790		1.576	6.94
X/2 Macrophyte	2.722	6.08	2.952	--	4.656	7.71	--		--		0.941	6.67
X Macrophyte	2.535	7.03	3.507	--	4.796	9.59	4.424	10.13	1.433		1.205	6.79
2X Macrophyte	1.897	8.17	--	--	4.141	9.72	--		--		1.405	6.61
X Macrophyte plus Na_2CO_3	3.464	7.47	4.179	7.41	4.787	9.77	5.095	10.24	--		0.922	6.88

Optimum Light

Macrophyte growth could be affected by the intensity, quality, and photoperiod of the available light. Because they grow submerged in water, macrophytes might respond to these light factors quite differently than do terrestrial species.

There were only limited opportunities for varying light quality in the artificially lighted growth chambers used in these studies. Some control was possible in the selection of fluorescent bulbs, which provided most of the light, and in the number of incandescent bulbs used to supplement radiation of long wavelengths. In the few variations attempted, there were no indications that any combination was superior to the ratio of fluorescent to incandescent light provided in the Sherer Growth Cabinets, Model CEL 25-7 HL, used in the current studies. Each chamber contained 6, cool-white, 4-foot fluorescent bulbs and 12, 25 watt incandescent bulbs. These are ratios considered suitable for crop plant growth under artificial light. There also was no indication that a light-dark cycle in each 24-hour period was superior to continuous light.

The effect of variations in light intensity on the growth of Elodea was investigated in an experiment summarized in Figure 4. The experimental setup was similar to that described in Section IV for establishing nutrient critical concentrations by the tray procedure. This procedure was used, because it permitted rapid evaluations of macrophyte response to light. One set of trays was maintained at approximately 1700 ft candles throughout the culture period; another set was exposed to different light intensities every several days during the culture period.

The results in Figure 4 compare the percent increase in length of Elodea in control trays exposed to continuous light of 1700 ft candles and in trays exposed to different light intensities at various stages of the culture period. The data indicate Elodea was not damaged by light intensities as high as 2600 ft candles; in fact, growth was slightly better at 2600 ft candles than at 1700. When light intensity was reduced to 710, 390, and 110 ft candles, growth was less than at 1700 ft candles and progressively less with each decrease in intensity.

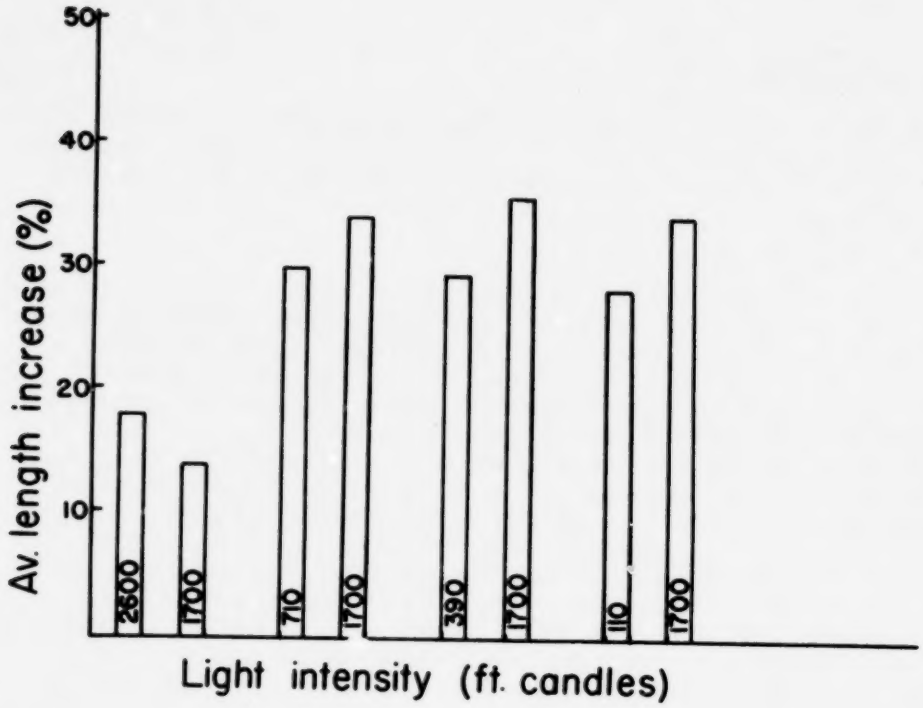

Figure 4. The growth of Elodea occidentalis, as length increase, during periods of exposure to various light intensities.

The limitations of using length increase as a criterion of growth must be recognized. Nevertheless, from the data obtained, maintenance of a specific light intensity does not seem critical in macrophyte culture. Elodea made reasonably good growth over the range of light likely to be available in artificially lighted growth chambers. On this project, light intensities were maintained in the range of 800-1700 ft candles.

As with terrestrial species, light intensity had a marked effect on plant morphology and color. At the lower light intensities, the Elodea was unusually dark green in color and the leaves were thinner and the internodes longer than in the plants grown at 1700 ft candles.

Optimum Temperature

The temperature of 23°C used for macrophyte culture on this project was established from the data presented in Figure 5. The growth of Elodea, as length increase

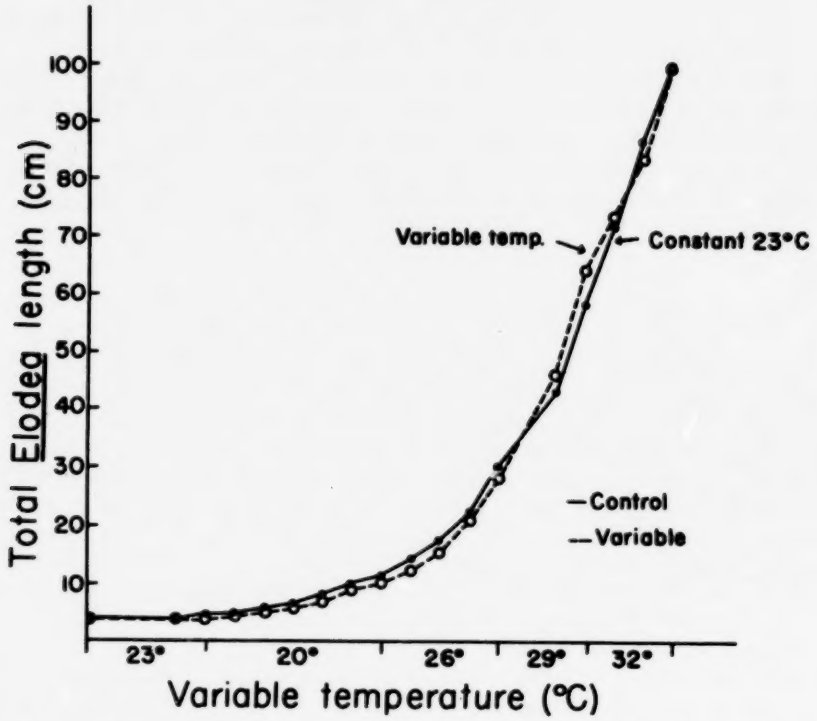

Figure 5. The growth of Elodea occidentalis, as length increase, during exposure to various temperatures.

was compared in a set of trays maintained at 23°C and in trays exposed to temperatures varying from 20 to 32°C during different phases of the culture period.

Growth was slightly less at 20°C than at 23°. There were were only slight differences in growth at 23°C and temperatures up to 32°C. It seems, therefore, that Elodea is not highly sensitive to temperatures within the 20 to 30°C range.

CO_2 Concentration

For many years, it has been common practice to bubble CO_2-enriched air through algae cultures. For unicellular green algae, 5% CO_2 has been widely used. There was reason, therefore, to question that the recommended 1% was the optimum CO_2 level for macrophytes under the culture conditions employed. In experiments which will not be reported in detail, CO_2 concentrations less than 0.4% resulted in a reduced rate of Elodea growth. The rate of growth was, however, not increased by raising the CO_2 concentration above 0.4%. The maximum concentration tested was 2.0% which was not inhibitory to the Elodea.

DISCUSSION

The nutrient medium for macrophytes developed in this study was demonstrated to produce excellent growth of several species. It is anticipated the medium will be equally suitable for other macrophytes. This optimism is based on general experience with plant culture media. Once the general features of a nutrient medium are established, it usually is satisfactory for many species. Species within a major taxonomic group are not sensitive to minor variations in nutrient solution composition.

Establishing techniques and conditions necessary for laboratory culture of macrophytes should stimulate basic studies on the neglected area of the nutrition and physiology of these organisms. This background information is critical for understanding the behaviour, distribution, and ecology of the macrophytes. Laboratory studies can be particularly useful in providing the basic data on macrophyte nutritional requirements needed in developing measures to control nuisance growths of these organisms.

SECTION VII

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SECTION VIII

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SECTION IX

APPENDIX

The concentrations of essential elements in first and second one-inch segments of *Elodea occidentalis* and *Ceratophyllum demursum* collected at intervals from various Wisconsin lakes during 1970.

Table		Page
A	Allequash Lake, *Elodea*	58
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F	Nebisch Lake, *Elodea*	63
G	Big Kitten Lake, *Elodea*	64
H	Big Kitten Lake, *Ceratophyllum*	65
I	Little John Lake, *Ceratophyllum*	66

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A. Allequash Lake, Elodea.

Date Sampled	Segment	% N	% P JAS	% P Std.	% S	% K	% Ca JAS	% Ca Std.	% Mg JAS	% Mg Std.	Ppm Fe	Ppm B	Ppm Cu JAS	Ppm Cu Std.	Ppm Zn	Ppm Mn	Ppm Mo
June 11	1"	3.71	0.47			2.10	0.47		0.22		>1000	18.1	4.7		123	70	
	2"	3.55	0.35			2.20	0.57		0.18		>1000	16.6	4.2		128	103	
July 6	1"	3.42	0.31			1.90	0.42		0.18		610	11.6	2.3		87	116	0.58
	2"	2.91	0.24			1.79	0.59		0.19		775	15.4	2.6		101	193	
July 27	1"	3.53	0.34	0.31		2.00	0.42	0.41	0.23	0.18	745	15.9	3.8	6.5	90	83	
	2"	3.19	0.28	0.22		1.90	0.61	0.54	0.21	0.14	>1000	18.8	3.4	7.0	118	168	
Aug. 18	1"	2.94	0.42		0.26	2.20	0.55		0.20		>1000	14.4	5.7		64	93	
	2"	2.82	0.35		0.29	2.30	0.80		0.22		950	17.0	6.1		88	158	

B. Clear Lake, Elodea.

Date Sampled	Segment	% N	% P JAS	% P Std.	% S	% K	% Ca JAS	% Ca Std.	% Mg JAS	% Mg Std.	Ppm Fe	Ppm B	Ppm Cu	Ppm Zn	Ppm Mn	Ppm Mo
June 18	1"	3.27	0.56	0.39		2.10	0.31		0.21		995	17.5	5.9	77	1000	0.54
	2"	2.67	0.51	0.32		2.65	0.44		0.20		>1000	21.6	5.5	95	1000	
July 8	1"	2.47	0.47	0.28		2.10	0.68		0.17		>1000	16.0	4.3	106	1000	0.73
	2"	2.28	0.33	0.24		2.35	0.73		0.17		>1000	14.3	2.3	90	1000	
July 29	1"	2.41	0.42	0.30	0.30	2.00	0.69	0.65	0.21	0.15	715	15.9	2.2	131	356	
	2"	1.94	0.28	0.24	0.28	2.35	0.66	0.80	0.16	0.14	665	14.8	1.8	103	398	
Aug. 20	1"	2.62	0.42	0.27		2.30	0.85		0.17		750	13.8	2.2	89	392	
	2"	2.03	0.38	0.27		2.75	1.05		0.17		555	11.3	<1.0	93	1000	

C. Little Spider Lake, Elodea.

Date Sampled	Segment	% N	% P JAS	% P Std.	% S	% K	% Ca JAS	% Ca Std.	% Mg JAS	% Mg Std.	Ppm Fe	Ppm B	Ppm Cu JAS	Ppm Cu Std.	Ppm Zn	Ppm Mn
June 15	1"	3.59	0.47			2.45	0.44		0.28		740	18.8	2.1		64	170
	2"	2.78	0.30			2.80	0.66		0.29		775	19.9	<1.0		80	268
July 7	1"	3.75	0.42			2.20	0.44		0.26		610	14.3	2.3		79	215
	2"	3.08	0.28			2.65	0.62		0.25		625	13.1	<1.0		80	290
July 28	1"	3.36	0.44	0.37	0.29	2.65	0.52	0.55	0.31	0.25	390	17.0	3.8	6.5	127	165
	2"	2.59	0.28	0.27	0.31	2.65	0.61	0.64	0.28	0.24	400	16.6	2.2	4.5	105	259
Aug. 19	1"	2.64	0.32			2.75	0.68		0.32		425	14.4	<1.0		108	198
	2"	2.29	0.23			2.90	0.85		0.30		345	13.8	<1.0		89	286

D. Erickson Lake, Elodea.

Date Sampled	Segment	%								Ppm					
		N	P		S	K	Ca		Mg		Fe	B	Cu	Zn	Mn	Mo
			JAS	Std.			JAS	Std.	JAS	Std.						
June 16	1"	3.96	0.54			2.80	0.36	0.33	0.24		>1000	17.0	7.9	99	96	0.59
	2"	3.40	0.39			3.10	0.37	0.36	0.18	0.16	>1000	18.1	4.1	77	121	
July 9	1"	3.70	0.44			2.35	0.32	0.28	0.21		695	11.4	2.6	114	80	
	2"	2.94	0.23			2.80	0.61	0.37	0.17	0.15	670	12.1	2.6	161	109	
July 28	1"	3.41	0.47	0.41	0.27	2.75	0.28	0.28	0.22	0.18	465	13.1	3.8	97	65	
	2"	2.70	0.29	0.28	0.28	2.75	0.44	0.40	0.18	0.14	440	15.9	2.2	145	98	
Aug. 19	1"	3.28	0.49			2.55	0.44	0.42	0.21		730	12.7	4.7	100	80	
	2"	2.59	0.34			2.75	0.59	0.52	0.18	0.15	450	12.7	2.2	78	65	
Sept. 8	1"	2.89	0.42			1.20	0.69	0.56	0.18		605	10.7	3.1	80	114	
	2"	2.15	0.29			1.54	0.78	0.72	0.19	0.16	365	9.4	1.5	79	116	

E. Whitney Lake, Elodea.

Date Sampled	Segment	N	P		S	K	Ca		Mg		Fe	B	Cu	Zn	Mn	Mo
		%									Ppm					
			JAS	Std.			JAS	Std	JAS	Std.						
June 18	1"	2.88	0.38			1.63	0.32	0.32	0.17	0.14	>1000	19.1	2.5	75	63	0.53
	2"	2.23	0.34			1.77	0.44	0.42	0.17	0.14	>1000	22.5	3.7	97	93	
July 8	1"	2.66	0.28			1.85	0.61		0.16		805	12.1	2.3	86	100	0.53
	2"	2.18	0.20			2.20	0.82		0.18		765	13.6	2.6	112	150	
July 29	1"	2.34	0.23	0.21	0.32	1.42	0.68	0.74	0.15	0.10	999	15.4	2.2	125	137	
	2"	2.02	0.21	0.17	0.42	1.76	0.89	1.02	0.17	0.15	790	11.1	1.8	141	155	
Aug. 20	1"	3.09	0.37			2.30	0.57		0.20		965	11.7	2.2	158	93	
	2"	2.52	0.33			2.65	0.87		0.19		965	14.4	2.2	174	126	
Sept. 9	1"	3.50	0.49			2.30	0.44		0.20		830	10.2	2.6	115	65	
	2"	2.98	0.39			2.65	0.64		0.18		890	13.1	<1.0	109	103	

F. Nebisch Lake, Elodea

Date Sampled	Segment	% N	% P JAS	% P Std.	% K	% Ca	% Mg	Ppm Fe	Ppm B	Ppm Cu	Ppm Zn	Ppm Mn
Sept. 10	1"	3.76	0.75	0.44	2.65	0.42	0.22	660	11.6	8.1	135	119
	2"	4.41	0.56	0.38	2.80	0.61	0.19	580	10.7	5.0	123	160

G. Big Kitten Lake, Elodea

Date Sampled	Segment	N	P		S	K		Ca		Mg		Fe	B	Cu		Zn	Mn	Mo
			%			%		%		%		Ppm	Ppm	Ppm		Ppm	Ppm	Ppm
			JAS	Std.		JAS	Std.	JAS	Std.	JAS	Std.			JAS	Std			
June 18	1"	3.11	0.49	0.38		2.55	2.97	0.87	0.81	0.23	0.18	685	15.4	2.5	5.3	67	>1000	0.50
	2"	2.74	0.44	0.27		2.80	3.15	1.11	0.90	0.22	0.16	595	17.5	2.5		133	>1000	
July 8	1"	3.84	0.56	0.50		2.45		0.66	0.73	0.22	0.22	305	10.9	2.3	4.5	89	223	
	2"	3.13	0.44	0.35		2.90	3.09	0.89	0.83	0.19	0.18	350	12.7	1.0		87	356	
July 29	1"	2.71	0.42	0.39	0.29	2.45	2.28	1.61	2.24	0.22	0.21	260	12.0	<1.0	3.9	74	313	
	2"	2.16	0.40	0.34		2.45		1.51	2.61	0.23	0.22	225	13.5	2.2	2.7	78	348	
Aug. 20	1"	2.62	0.38	0.34		2.30	2.50	1.69	1.80	0.23		400	11.6	<1.0	1.8	55	240	
	2"	2.13	0.34	0.33		2.45	2.54	2.03	2.59	0.25		295	12.2	<1.0		43	290	
Sept. 9	1"	2.54	0.44	0.34		1.85	2.15	2.41	2.09	0.22		260	5.3	<1.0	1.9	64	348	
	2"	2.35	0.51	0.35		2.00	2.01	2.01	3.51	0.23		180	7.1	<1.0		58	333	

H. Big Kitten Lake, Ceratophyllum

Date Sampled	Segment	N (%)	P (%)		S (%)	K (%)		Ca (%)		Mg (%)		Fe (Ppm)	B (Ppm)	Cu (Ppm)		Zn (Ppm)	Mn (Ppm)
			JAS	Std.		JAS	Std.	JAS	Std.	JAS	Std.			JAS	Std.		
July 29	1"	3.48	0.42	0.36	0.37	4.05	4.62	0.15	0.27	0.61	0.66	200	17.5	<1.0	3.1	63	>1000
	2"	2.48	0.23	0.23		4.35		0.28		0.64		245	17.5	<1.0	2.7	87	>1000
Aug. 20	1"	2.45	0.28	0.28		3.45		0.29	0.68	0.83		255	13.8	<1.0	2.2	33	368
	2"	1.86	0.19	0.19		3.45	4.19	0.37	0.35	0.72	0.83	345	14.4	<1.0		24	>1000
Sept. 9	1"	2.48	0.42	0.27	0.27	3.45	4.14	0.32	0.29	0.72		140	14.6	1.5	2.9	44	374
	2"	1.81	0.29	0.19	0.26	3.65	4.03	0.31	0.28	0.78		150	14.6	<1.0		39	>1000

I. Little John Lake, Ceratophyllum

Date Sampled	Segment	% N	% P JAS	% P Std.	% S	% K JAS	% K Std.	% Ca JAS	% Ca Std.	% Mg JAS	% Mg Std.	Ppm Fe	Ppm B	Ppm Cu JAS	Ppm Cu Std	Ppm Zn	Ppm Mn
June 10	1"	3.48	0.58			3.00		0.24		0.70		850	17.5	3.0		79	>1000
	2"	2.84	0.56	0.35		2.45	2.74	0.42	0.33	0.85		>1000	28.3	5.6	4.4	96	>1000
July 6	1"	2.73	0.51	0.38		3.65	3.69	0.10	0.19	0.64		415	14.9	1.45	4.1	49	>1000
	2"	2.35	0.51	0.33		2.90	3.50	0.21	0.25	0.73	0.77	520	16.3	1.00		49	>1000
July 27	1"	2.14	0.30	0.24	0.19	3.10	3.59	0.13	0.21	0.64	0.56	525	13.5	<1.0	1.0	32	>1000
	2"	1.86	0.30	0.20	0.20	2.75	3.66	0.17	0.24	0.78	0.72	660	13.5	<1.0	1.5	41	>1000
Aug. 18	1"	2.27	0.34	0.28		2.65	3.44	0.14	0.26	0.50		685	13.8	1.4	3.8	28	>1000
	2"	1.96	0.30	0.21		2.75	2.96	0.16	0.23	0.64	0.62	540	12.7	<1.0	2.8	30	>1000
Sept. 8	1"	3.00	0.75	0.49	0.29	3.25	4.23	0.23	0.27	0.80		925	18.9	1.5	2.0	49	>1000
	2"	2.35	0.61	0.35	0.28	3.00	3.96	0.24	0.28	0.78		>1000	18.9	1.5		46	>1000

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